American Museum Novitates

PUBLISHED BY THE AMERICAN MUSEUM OF NATURAL HISTORY
CENTRAL PARK WEST AT 79TH STREET, NEW YORK 24, N.Y.

NUMBER 1999 MARCH 29, 1960

Hybridization in Two Sympatric Species of Atherinid Fishes, *Menidia menidia* (Linnaeus) and *Menidia beryllina* (Cope)[1]

BY IRA RUBINOFF[2] AND EVELYN SHAW[3]

The genus *Menidia* consists of four species: *M. menidia* (Linnaeus), *M. beryllina* (Cope), *M. audens* Hay, and *M. extensa* Hubbs and Raney (Gosline, 1948). *Menidia menidia* ranges from Nova Scotia to Florida. *Menidia beryllina* has a more southern distribution, extending from Massachusetts to Veracruz, Mexico. The fresh-water species *M. audens* is found in the lower Mississippi Valley and as far inland as Oklahoma (Moore and Cross, 1950). *Menidia extensa* is reported only from Lake Waccamaw in North Carolina (Hubbs and Raney, 1946).

Two species of silversides, *M. menidia* and *M. beryllina,* occur sympatrically throughout most of the eastern United States coastal waters, and natural hybrids have been reported from certain rivers in northeastern Florida (Hubbs and Raney, 1946; Gosline, 1948). Analysis of collections taken at Woods Hole, Massachusetts, indicates that the populations apparently are discrete, with no indications of natural hybridization in the series examined.

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[1] The laboratory facilities at the Marine Biological Laboratory, Woods Hole, Massachusetts, were provided by the Marine Biological Laboratory through their contract with the Office of Naval Research.

[2] Biological Laboratories, Harvard University.

[3] Research Associate, Department of Animal Behavior, the American Museum of Natural History.

TABLE 1

DATA ON COLLECTIONS OF *Menidia* IN SUMMER, 1958

Species and Locality	Vertebrae Total			Vertebrae Caudal			Anal Rays			Standard Length		
	N	Mean	Range	*N*	Mean	Range	*N*	Mean	Range	*N*	Mean	Range
Sippewissett, Mass.												
menidia	16	43.5	42–45	16	25.1	23–26	16	23.3	22–26	16	80.8	72–90
Bowen Pond, Mass., coll. 1												
beryllina	36	38.2	37–39	36	21.6	21–23	51	15.5	14–17	51	46.8	40–57
Stony Beach, Mass.												
menidia	—	—	—	—	—	—	30	23.4	22–27	30	83.5	73–91
Great Pond, Mass.												
beryllina	—	—	—	—	—	—	9	15.1	14–16	9	44.0	42–46
Orleans, Mass., coll. 1												
menidia	—	—	—	—	—	—	38	23.7	20–26	38	82.5	56–111
beryllina	—	—	—	—	—	—	25	15.5	14–17	25	50.9	47–55
Bowen Pond, Mass., coll. 2												
menidia	—	—	—	—	—	—	1	22	—	1	80	—
beryllina	—	—	—	—	—	—	36	15.6	14–18	36	46.6	38–54
Bowen Pond, Mass., coll. 3												
menidia	—	—	—	—	—	—	7	23.8	22–29	7	78.5	67–97
beryllina	—	—	—	—	—	—	116	15.9	14–19	116	47.0	42–56
Orleans, Mass., coll. 2												
menidia	—	—	—	—	—	—	8	23.3	21–25	8	79.5	72–86
beryllina	—	—	—	—	—	—	14	15.7	14–17	14	50.6	46–59

TABLE 1—(*Continued*)

Species and Locality	Vertebrae						Anal Rays			Standard Length		
	Total			Caudal								
	N	Mean	Range	*N*	Mean	Range	*N*	Mean	Range	*N*	Mean	Range
Jamaica Bay, N. Y.												
menidia	—	—	—	—	—	—	34	23.4	21–25	34	77.8	58–99
TOTALS												
menidia	—	—	—	—	—	—	134	23.5	20–29	134	80.9	56–111
beryllina	—	—	—	—	—	—	251	15.6	14–19	251	47.1	38–59

The present study attempts to determine whether the genetic constitution of the two species of *Menidia* is sufficiently dissimilar to prevent genetic interchange under artificial conditions. As part of the analysis, artificial hybridization was undertaken. If it proved successful, the morphology of the artificially produced hybrids would then be a useful guide in the identification of hybrids occurring under natural conditions.

ANALYSIS OF COLLECTIONS

An analysis of the collections of *Menidia* taken from various sites in the Cape Cod area is presented in table 1. The majorities of these collections were made at sites where *M. menidia* and *M. beryllina* occur sympatrically.

The morphological features which were most useful in the separation of the species were: the number of rays in the anal fins, the standard lengths, the vertebral counts, and the total number of lateral scales. Figure 1 shows the bimodal distribution occurring when anal

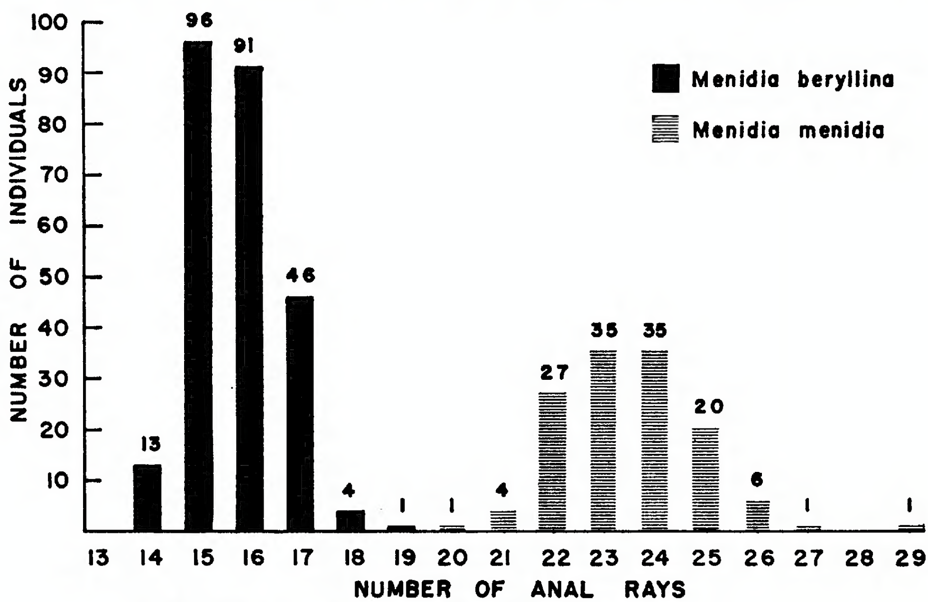

FIG. 1. Frequency of anal fin rays in all collections.

fin rays are counted; the range for 251 specimens of *M. beryllina* was 14–19, mean 15.6, and the range for 134 specimens of *M. menidia* was 20–29, mean 23.5. The two specimens showing 19 and 20 anal fin rays were classified as *M. beryllina* and *M. menidia*, respectively, on the basis of their standard lengths and the total number of their lateral

scales. The specimen of *M. beryllina* with 19 anal rays was 54 mm. long and had 38 scales in its lateral series. The specimen of *M. menidia* was 91 mm. in length and contained 45 scales in its lateral series. On the basis of these characteristics these two fish were not considered as hybrids.

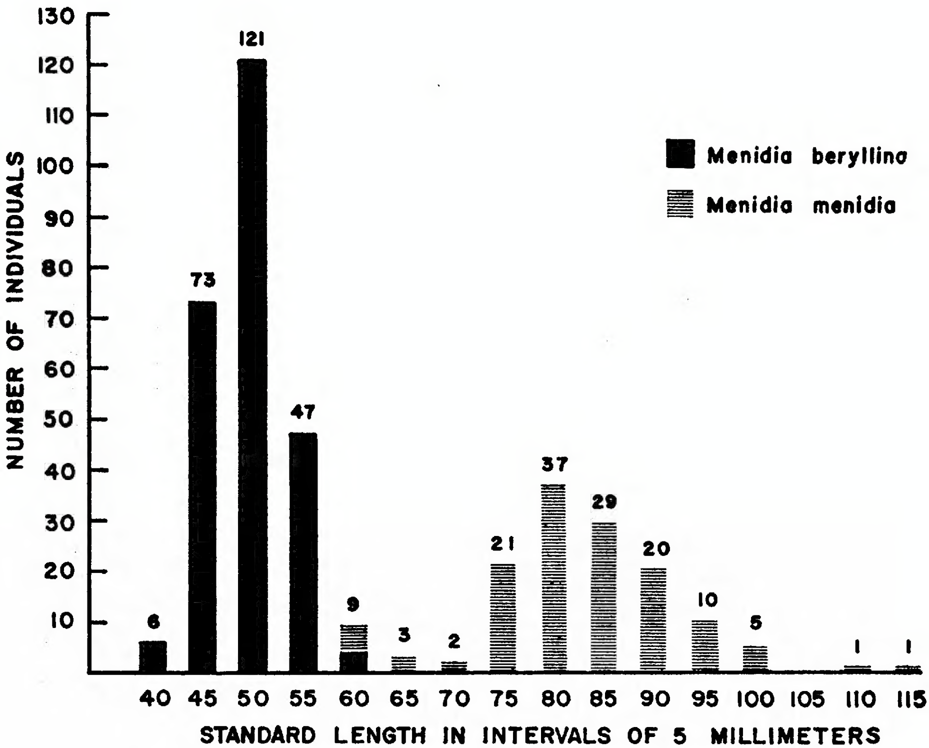

FIG. 2. Frequency of standard lengths in all collections.

Figure 2 shows a similar bimodal distribution of the standard lengths. *Menidia menidia* is the larger of the two species, mature fish ranging from 56 to 111 mm. Adult specimens of *M. beryllina* range from 38 to 59 mm. in standard length. Some specimens of *M. menidia* were smaller than the largest of *M. beryllina*, thereby causing the slight overlap.

The distance from the snout to the first dorsal fin as a percentage of the standard length is presented in figure 3. The ratio of these characters for both species was the same, and therefore was not useful in separating the two species. An analysis of many characters of these species produces similar overlaps, creating some difficulty in our identifying them.

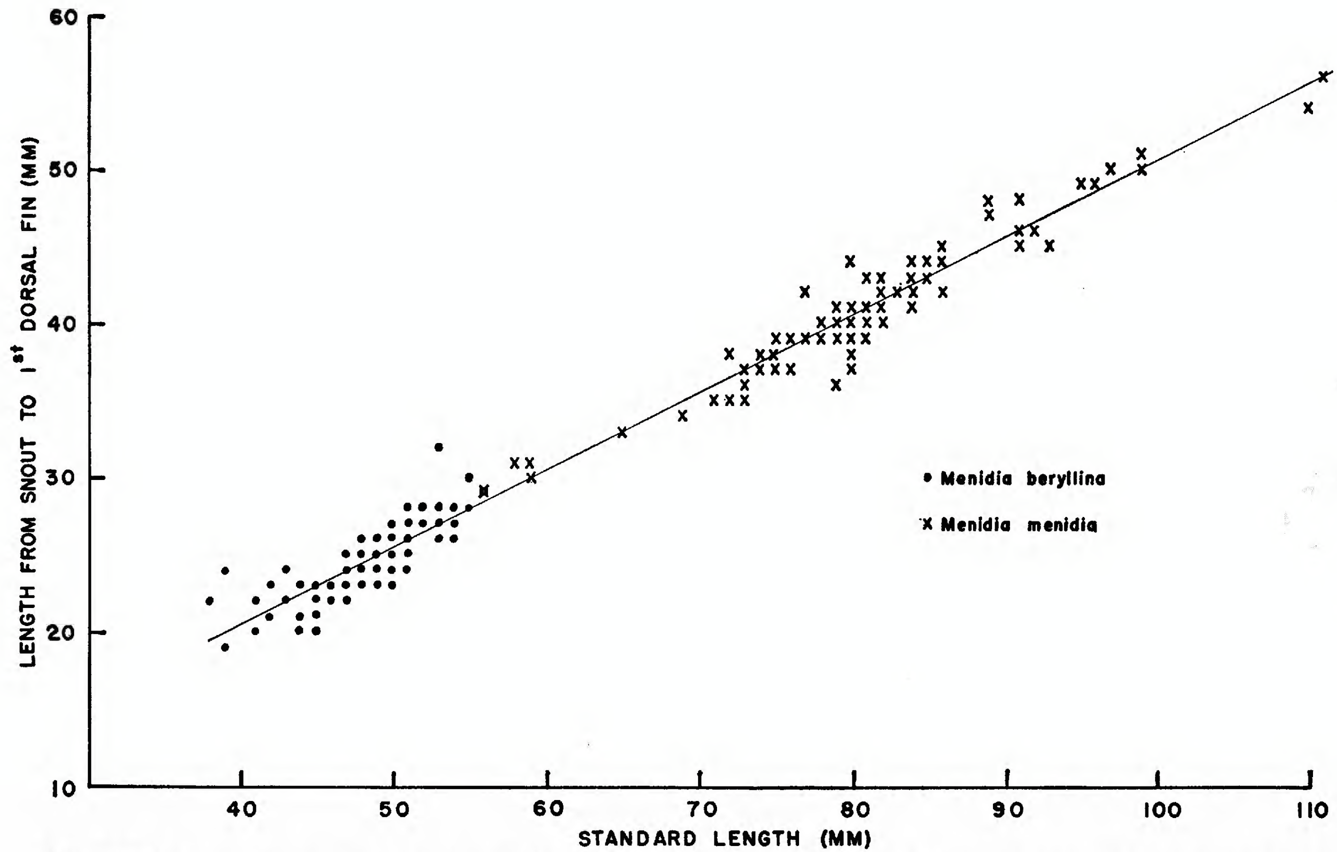

FIG. 3. Distance from snout to first dorsal fin as a percentage of standard length in 80 adult *Menidia menidia* and 80 adult *Menidia beryllina*.

MATERIALS AND METHODS

No difficulty was encountered in obtaining viable gametes; individuals of both species were ripe during most of June and July, 1958. The criteria for identification of the species were primarily size and the number of rays in the anal fins. Because there was no overlap in the number of anal rays in the sample used to provide the gametes for the experimental crosses, this character was the most convenient one for use in the separation of the two species. Analysis of the morphological characters of the specimens of *Menidia* used to furnish the gametes for the experimental crossings is presented in table 2.

Eggs and sperm from 16 adult specimens of *M. menidia* and 51 of *M. beryllina* were easily stripped from the ripe adults into separate

TABLE 2

SPECIMENS USED TO FURNISH GAMETES FOR HYBRIDIZATION: *Menidia menidia* FROM SIPPEWISSETT, MASSACHUSETTS; *Menidia beryllina* FROM BOWEN POND, MASSACHUSETTS

	menidia	*beryllina*
Standard length		
Size of sample	16	51
Mean	80.8	46.8
Range	72–96	40–57
Distance from snout to first dorsal as percentage of standard length		
Size of sample	16	51
Mean	50.4	48.4
Range	48–52.2	45.2–54
Second dorsal		
Size of sample	16	51
Mean	9.6	9.5
Range	8–10	9–11
Total number of scales		
Size of sample	16	51
Mean	46.6	39.2
Range	44–48	36–43
Anal rays		
Size of sample	16	51
Mean	23.3	15.5
Range	22–26	14–17
Total vertebral count		
Size of sample	16	36
Mean	43.3	38.2
Range	42–45	37–39

finger bowls. The gametes thus obtained were established in the following crosses:

HYBRIDS

M. menidia female × *M. beryllina* male
M. beryllina female × *M. menidia* male

CONTROLS

M. menidia female × *M. menidia* male
M. beryllina female × *M. beryllina* male

The eggs were allowed to remain in the appropriate sperm solution for 12 minutes before they were rinsed with filtered sea water and then incubated in finger bowls, 4 inches in diameter, of sea water at room temperature (eggs handled similarly but without the sperm solution did not develop). Approximately one-quarter of the sea water in each bowl was siphoned off every other day and replaced with fresh sea water. After hatching, the larvae were transferred to finger bowls, 7 inches in diameter, and the procedure of water changing was continued.

The *Menidia* embryos began to hatch nine days after fertilization, and they were immediately fed the newly hatched larvae of the brine shrimp, *Artemia* (Rubinoff, 1958). Four weeks after hatching it was necessary to terminate these experiments. At this time most of the young had differentiated sufficiently for accurate counts of the number of rays in the anal fins to be made.

The method of counting the anal rays used in the present paper is identical with that described by Gosline (1948). The first full-length anal ray is considered the first; the last two rays which emerge from the same proximal base are considered as one. The standard length was measured from the anterior edge of the upper jaw to the end of the vertebral column.

The small size of the experimental crosses precluded the use of the standard technique of counting caudal vertebrae by X-ray photography. Instead, these fishes were treated with 3 per cent potassium hydroxide for 24 hours, stained with alizarin red and cleared with glycerine. Although alizarin red was not an ideal stain for fish of this size, it was possible to make accurate counts of the vertebrae, starting from the vertebra adjacent to the anterior edge of the anal fin to the urostyle.

EXPERIMENTAL RESULTS

Growth and development and the time of hatching were identical with those of the control groups. Approximately 200 embroyos began

developing in each of the four groups. The number of survivors is not, however, an indication of the viability of survival rate, as many of the embryos were periodically preserved for histological study. No differences could be detected, as an indication of hybrid inferiority or vigor, between the growth rate and the survival rate of the groups.

The characteristics of the reciprocal hybrid crosses and the straight cross controls are presented in table 3. When the experiments were terminated a total of 45 hybrids, 8.5–15 mm. in length, were differentiated sufficiently to permit accurate counts of the anal rays; fins of the 12 other surviving hybrids were not sufficiently differentiated to permit counts. Among the controls, 32 fish, 9–15 mm. in length, could be counted, while 29 were still undifferentiated.

TABLE 3

RESULTS OF STRAIGHT CROSS CONTROLS IN *Menidia* AND OF RECIPROCAL HYBRID CROSSES
(Fertilized, July 3, 1958; hatched, about July 13, 1958; preserved, August 6, 1958.)

	No. of Rays in Anal Fins	Standard Length	No. of Caudal Vertebrae
menidia ♀ × *menidia* ♂			
Total number of specimens	41	41	41
Number of specimens measured	15	14	8
Mean	22.8	10.6	25.2
Range	22–25	9.0–15.0	25–26
beryllina ♀ × *beryllina* ♂			
Total number of specimens	20	20	20
Number of specimens measured	17	17	12
Mean	15.8	10.4	22.3
Range	14–17	9–12	20–24
beryllina ♀ × *menidia* ♂			
Total number of specimens	16	16	16
Number of specimens measured	16	16	14
Mean	18.6	10.3	24.1
Range	17–20	9–11.5	24–25
menidia ♀ × *beryllina* ♂			
Total number of specimens	41	41	41
Number of specimens measured	29	29	10
Mean	20.8	10.2	25.3
Range	19–23	8.5–15	25–27

A comparison of the distribution and frequency of the anal fin rays of the hybrids with those of the parents indicates that in both reciprocal cases the hybrids were intermediate between the parents. However,

it should be noted that there is an apparent maternal influence in the determination of the number of anal fin rays (table 4). The means and ranges of the anal fin ray distribution in the hybrids more closely resemble those of the female parent than those of the male. *Menidia beryllina* females × *M. menidia* males produced progeny with a mean of 18.7 rays in their anal fins which was closer to the mean of 15.5 of

TABLE 4

FREQUENCY OF THE NUMBER OF ANAL FIN RAYS IN *Menidia menidia*, *Menidia beryllina*, AND THEIR HYBRID AND STRAIGHT CROSS OFFSPRING

	Number of Rays													Total No. of Fish
	14	15	16	17	18	19	20	21	22	23	24	25	26	
Parents														
menidia	—	—	—	—	—	—	—	—	6	3	4	3	—	16
beryllina	4	19	19	9	—	—	—	—	—	—	—	—	—	51
F_1 hybrids														
beryllina ♀ × *menidia* ♂	—	—	—	2	5	5	4	—	—	—	—	—	—	16
menidia ♀ × *beryllina* ♂	—	—	—	—	—	7	6	5	6	5	—	—	—	29
F_1 controls:														
menidia ♀ × *menidia* ♂	—	—	—	—	—	—	—	—	7	5	2	1	1	16
beryllina ♀ × *beryllina* ♂	2	3	7	5	—	—	—	—	—	—	—	—	—	17

M. beryllina than it was to the parental mean of 23.3 anal rays in *M. menidia*. The mean number of anal rays of the reciprocal cross *M. menidia* females × *M. beryllina* males was 20.8, much closer to the *M. menidia* parent.

DISCUSSION

Reciprocal hybrid crosses were found to be perfectly viable for at least 25 days, post-hatching, under laboratory conditions. Whether or not the hybrids could have survived and developed into fertile adults is not known. If they did prove to be fertile, they would provide, potentially, a gene pool, which may ultimately alter the genotype of one or of both species. If the hybrids were sterile, they would provide an effective isolation barrier.

However, we know that hybrids can be produced, that they were found in Florida under natural conditions, but that no hybrids were found in the populations analyzed from an area around Woods Hole,

Massachusetts. The question is raised as to what isolation mechanisms may be operating to keep these species discrete in this area. Several possible isolation mechanisms may be working either alone or in combination.

Selection of a Species-specific Spawning Site

Very little is known about the actual spawning of *Menidia*. Isolation may be effected by the parental selection of specific spawning sites. It has been reported that under certain conditions the water in which *M. beryllina* spawns is less saline than the water in which *M. menidia* spawns. The reports of Bumpus (1898) and Nichols and Breder (1927) seem to indicate a promiscuous type of spawning. By either locating these fish during spawning, or by collecting their eggs, which are slightly different morphologically (Hildebrand, 1922), it would be possible to determine whether or not there is spawning site selection.

Sperm Specificity

In the absence of other reproductive isolating mechanisms, gametic isolation may play a role. Clark, Aronson, and Gordon (1954) introduced 1:2 mixtures of homospecific and heterospecific sperm of platyfish and swordtails into virgin female platyfish. The resulting progeny rarely contained hybrids, even though there was a greater number of hybrid-producing sperm present in the females. It is possible to interbreed *Menidia* in the laboratory, but under natural conditions, when a more diluted concentration of sperm is presented, there may be a mechanism whereby homospecific sperm are selected and gametic isolation may play a role.

Population Density

Gosline (1948) suggested that hybridization may occur when the numbers of a species become too low for normal spawning, as may be the case of *M. menidia* at its southern extension. This situation was not found in the Woods Hole area, where *M. menidia* and *M. beryllina* were both in abundance. However, that low numbers of one species results in interspecific crosses is questioned. If Gosline's hypothesis is correct, then hybridization would also be expected to occur in similar cases of lowered densities of one of the species, as is the situation in rivers where there is frequently a salinity-determined gradation of the two populations. Cursory examinations of collections from brackish portions of a river have not as yet revealed any hybridization.

Schooling Behavior

It is known that individuals in a school are frequently about the same size. The size difference between *M. menidia* and *M. beryllina* is such that they may swim in separate schools at all times. Casual observation in shoal waters revealed *Menidia* schools composed of only a single species, although during seining operations both species were often found in the same haul. It is not known whether the fish were all members of the same school, as the net was 50 feet long and was hauled through large areas.

A curious phenomenon has been observed in that, frequently, collections are made that are composed almost totally of members of the same sex. On one occasion, in an examination of over 200 adult *M. beryllina*, only 12 males were found. Kendall (1901) and Hildebrand (1922) also reported this phenomenon. Possibly the sexes may be separated during much of the breeding season, coming together only when certain specific ecological conditions are satisfied. Field studies will be continued, with the hope of our actually witnessing the spawning behavior of the species and also to learn whether or not schooling is restricted to conspecific individuals.

CONCLUSIONS

1. Successful hybridization between *M. menidia* and *M. beryllina* is possible, thus experimentally verifying Gosline's observations.
2. Hybrids are divisible into two maternally influenced phenotypes.
3. Although hybridization is possible, it is rare in nature, presenting the problem of which mechanisms effect the isolation.
4. The genus *Menidia* represents excellent material for future investigations of speciation in marine organisms.

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